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WITNESS my hand this
Fifth day of October 2000

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AUSTRALIA

Patents Act 1990

Proteome Systems Ltd

PROVISIONAL SPECIFICATION

Invention Title:

Electrophoresis Apparatus and a Method of using the Same

The invention is described in the following statement:

Field of the Invention

This invention relates to an electrophoresis apparatus and to a method of using the same for separating biomolecules by electrophoresis.

Background of the Invention

Two-dimensional electrophoresis is the preferred method for separating proteins from complex mixtures such as tissue samples, bacteria or plant material. Typically the proteins are separated in the first dimension using an electrophoresis gel with an immobilised pH gradient (IPG). These gels are commercially available and are usually supplied as dry gel strips bonded to a plastic backing sheet. Before the separation takes place the gel must be rehydrated with an appropriate liquid, in which it is ideal to have the protein sample dissolved. The most common embodiment of this approach is to allow the rehydration to occur passively, in a tray comprising a plurality of troughs, until the liquid in each trough has all be taken up by the IPG gel strip in that trough. This requires that care is taken in selecting the correct volume of rehydration liquid to match the capacity of the IPG gel strip. If too little rehydration liquid is added the IPG will under-rehydrate and the separation will be compromised. Similarly, if too much rehydration liquid is added and some of that liquid is not taken up by the IPG gel strip then proteins are lost in the liquid which is not taken up into the gel. Typically, high molecular weight proteins are preferentially lost in this process.

To overcome this drawback with passive rehydration some groups have advocated the use of rehydration trays with electrodes embedded in the troughs. The electrodes are used to provide a voltage (~50V) during the rehydration process. This electric field causes 'active' transport of the proteins into the IPG gel matrix and results in more proteins entering the gel, especially high molecular weight proteins. However, if the rehydration solution comes in contact with both electrodes during the rehydration process, then the dissolved proteins may undergo electrophoretic transport to the electrodes in the free solution. If this occurs, a significant proportion of the proteins of the sample do not separate in the IPG because the sample proteins are transported to the electrode and then precipitate there. The proteins which are lost in this process represent all molecular weights, not only high molecular weight proteins.

The present invention aims to provide an IPG gel strip rehydration tray that allows active rehydration to be done without the free rehydration solution coming into contact with the electrodes, thus preventing the electrophoretic transport of the proteins to the electrodes.

Disclosure of Invention

In a first aspect of the present invention there is provided an apparatus for rehydrating and for performing electrophoresis on a gel strip including:

(a) a tray defining at least one trough configured to receive a gel strip, said trough defining a centrally located rehydration area and an electrode area disposed either side of the electrode area;

(b) means for delimiting the rehydration area of the trough from the electrode area; and

(c) electrode means including contact points adapted to contact either the gel strip in the electrode areas near the first and second end of the gel or a conducting or current carrying electrode bridge material which is in contact with the gel strip in the electrode areas, the electrode means being adapted to be connected to a means for supplying an electric current for imposing an electric potential in the strip between the electrodes.

Existing apparatus for rehydrating IPG gel strips all have troughs with flat bases or floors. Indeed the provision of a flat floor in troughs for rehydrating IPG gel strips is taught as being necessary for satisfactory rehydration. In contrast the inventors of the present invention have realised that having a floor or base in which the dehydration area is delimited from the electrode area by for example having a stepped floor or a wall or both. In such a manner active rehydration can be carried out without the rehydration solution coming into contact with the electrodes, thus preventing the electrophoretic transport of the proteins to the electrodes in free solution.

In a preferred embodiment means of preventing the rehydration solution from contacting the electrodes include small walls extending across the width of the trough and a relatively small air gap between the electrodes and thewalls.

In a further preferred embodiment the gel in the rehydration area of the trough contacts a conducting/current carrying, electrode bridge which completes the circuit to contact point of the electrodes.

The electrode bridges may comprise filter paper or the like wetted with an electrically conducting liquid.

It is preferred that the electrode area is deeper than the rehydration area.

Typically the tray will define a plurality of substantially parallel troughs.

The trays may be designed to allow electrodes to contact the gel/electrode bridge assembly from above, thus eliminating the need for embedded electrodes in the troughs.

This arrangement lends itself to a disposable IPG gel strip rehydration and running tray. The ability to use a disposable, combined IPG gel strip rehydration and running tray overcomes a number of drawbacks with other commercially available systems.

The trays may be supplied with the dry IPG gel strips and dry electrode bridge material already in place in the grooves, thus eliminating the major handling step of setting up the trays. In addition, with disposable trays there is no problem with carryover from one sample to the next, whereas the current commercial trays require careful washing between uses.

The electrode assembly may include moulded pressure points, which rest on the gel strip where it overlaps the electrode bridge to ensure a good electrical contact between the gel strip and the electrode bridge.

The invention also encompasses a method of rehydrating and performing electrophoresis on a gel strip using the apparatus according to the present invention and/or its preferred embodiments.

In a related aspect the invention provides a method of rehydrating and performing electrophoresis on a gel strip comprising the steps of:

(a) providing a tray defining at least one trough with a gel strip, located in said trough, the trough defining a centrally located rehydration area and an electrode area disposed either side of the electrode area in which an absorbent electrode bridge is provided, the trough including means for delimiting the rehydration area of the trough from the electrode area;

(b) wetting the bridges with an electrically conducting liquid;

(c) adding rehydration liquid, containing a sample to be separated by electrophoresis to the trough;

(d) inserting a dry gel strip into the trough if a gel strip is not already present in the trough, the gel strip being longer than the rehydration area so that its ends rest on the electrode bridges;

(e) applying relatively low voltage across the gel strip the during a first period in which rehydration of the gel strip occurs;

(f) subsequently applying a relatively higher voltage to perform electrophoresis on the sample.

Typically the sample will be a mixture of macromolecules such as proteins, although other samples containing DNA, RNA, amino acids or other components which can be separated by electrophoresis may be used.

Brief description of the Drawings

The invention will now be described, by way of example only, and with reference to the accompanying drawings in which:-

Figure 1 is a schematic view of an apparatus for active rehydration of IPG gels;

Figure 2 shows an enlarged view of an electrode bridge area of the apparatus of Figure 1;

Figure 2a is a schematic side view of walls which delimit a rehydration area of the apparatus; and

Figure 3 illustrates an electrode assembly inserted into the electrode bridge area of the apparatus.

Detailed Description of the Presently Preferred Embodiments

Turning now to the drawings, Figure 1 shows an embodiment of an apparatus 10 for rehydrating dry IPG gels with the aid of an applied electric field. In the embodiment shown in Fig. 1 the tray has ten elongate parallel grooves or troughs 12. However the tray could have more or less than ten grooves. The grooves shown in Figure 1 are 6mm wide, however in other embodiments the grooves they may be relatively narrower or relatively wider than 6mm.

Figure 2 shows an expanded view of one end of the tray in Figure 1. Each groove 12 has a base or floor 14 which is stepped at each end. Figure 2 shows one end of each groove however both ends of each of the grooves are substantially identical. Each groove defines a central rehydration area 16 at each longitudinal end of which there is a wall 18 which serves to contain the

rehydration fluid within the designated rehydration area 18. The wall is 1mm above the floor of the rehydration area. There is an electrode bridge region 20 on the opposite side of each wall 18. The electrode bridge region is 1mm below the floor of the rehydration area. The electrode bridge area may typically be 20mm long and contains an air gap 22 between the wall 18 which retains the rehydration solution. This prevents capillary movement from the rehydration area onto the electrode bridge region 20. The dimensions given above are however exemplary only and might be varied.

The grooves which are typically 6mm wide allow room for loading the rehydration solution when an IPG gel strip is already in place in the groove, such as in the disposable tray format discussed above. Standard commercially available IPG gel strips are 3.3mm wide, thus the 6mm wide grooves will also allow for the use of relatively wider non-standard IPG gel strips up to approximately 5mm wide.

The length of the grooves/trays may vary depending on the length of commercially available IPG gel strips which are to be used in the tray. Commercially available IPG gel strips are usually 7, 11, 13, 17 or 18cm long.

The rehydration area may be approximately 5mm shorter than the respective IPG gel strip to be rehydrated, to allow overlap of the IPG gel strip into the electrode bridge area. The length of the electrode bridge area is 20mm. In a preferred embodiment the electrode bridge area contains a 6mm X 20mm piece of 2mm thick filter paper which fills the electrode bridge area except for a small air gap 22. A piece of filter paper that size requires between 50 and 200µL of water to become slightly hydrated. The quantity of water in the electrode bridge area requires precise control, to allow electrical contact without excessive wetness, which would cause a disturbance in the separation.

In other embodiments of the design the electrode bridge area and/or the rehydration area could be scaled up or down in size to accommodate different requirements.

Figure 3 shows an expanded view of one end of the tray in Figure 1 illustrating an external electrode assembly 24 being lowered into the electrode bridge areas of the grooves.

The external electrode assembly 24 consists of a series of pressure blocks 26 (which are dark grey in Figure 3) which rest/press on the IPG gel strip to ensure good contact between the IPG gel strip and the electrode

bridge material and an electrode itself 28 (which is shown in light grey in Figure 3). The electrode element comprises a series of electrode elements 30 located at the end of each pressure block which are integral with a joining element 32 linking the electrode elements together. The electrode elements are located near the outer edge of the trough to make full use of the length of the electrode bridge material.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

Dated this day of

PROTEOME SYSTEMS LTD
Patent Attorneys for the
Applicant:

F.B. RICE & CO.

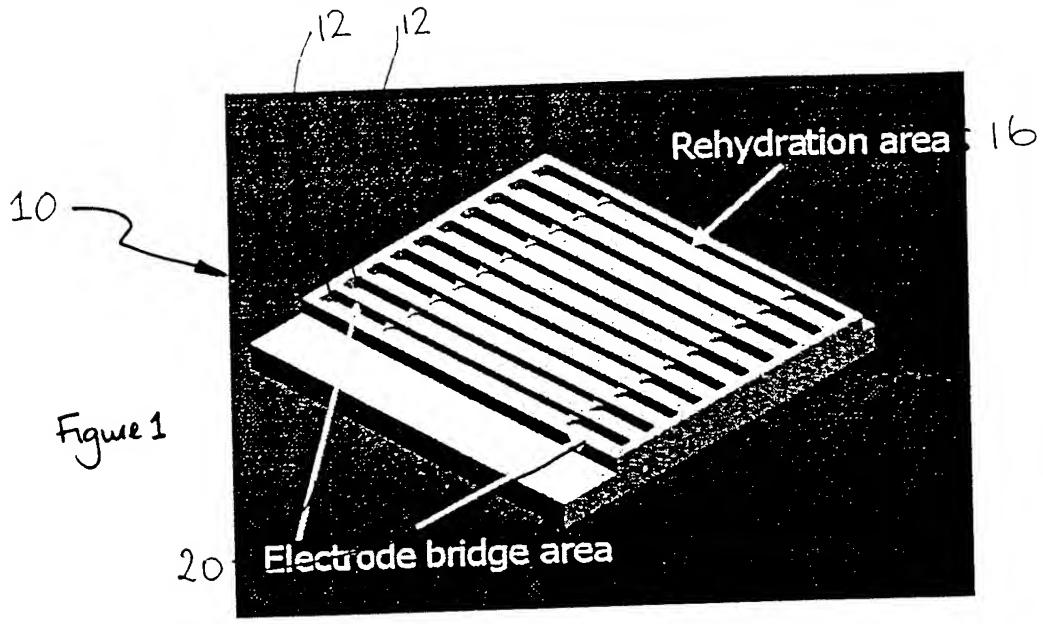


Figure 1

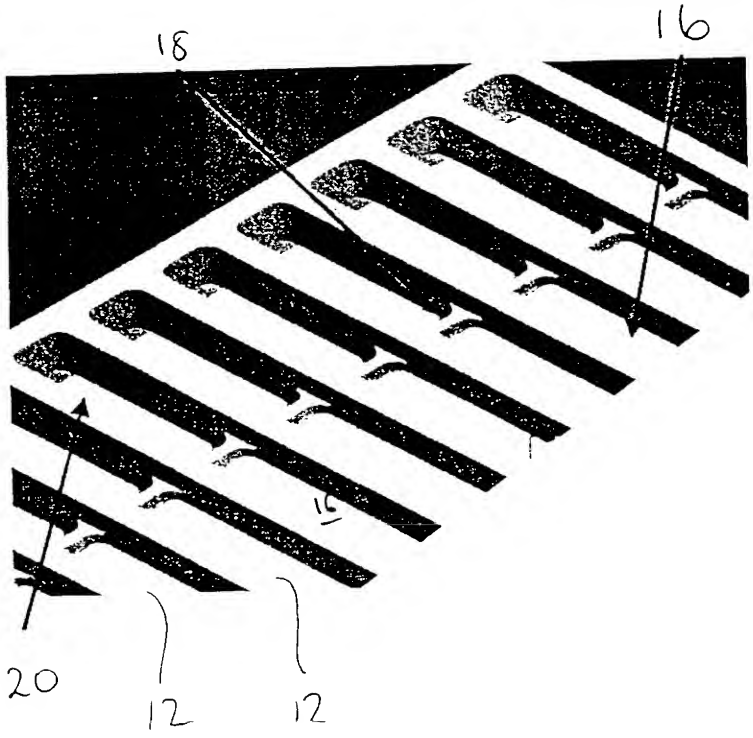


Figure 2

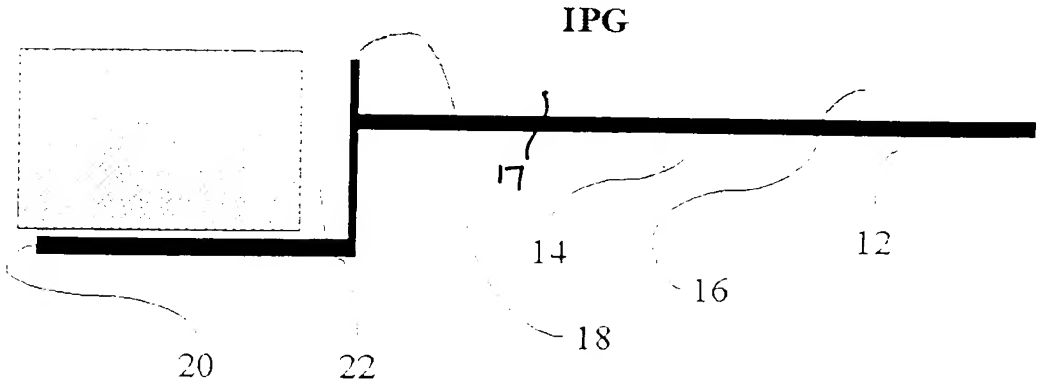
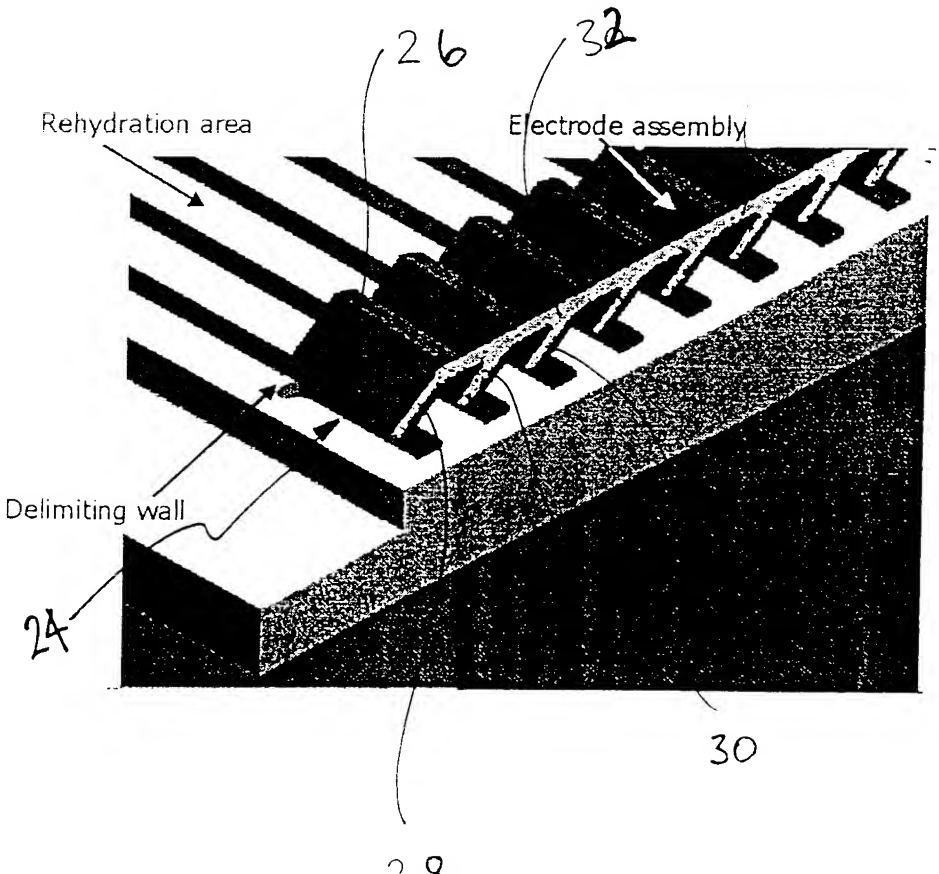


Fig 2a


26
32
Rehydration area
Electrode assembly
Delimiting wall
24
30
28